

Dietary Preference and Digestive Enzyme Activities as Indicators of Trophic Resource Utilization by Six Species of Crab

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Abstract. The digestive physiology and stomach contents of six crab species from a variety of habitats were investigated to provide an indication of their digestive capability and dietary preferences. Stomach contents varied between species, but the key enzymes present were generally consistent with the types of dietary material being ingested. *Nectocarcinus integrifrons* (red rock crab) consumed large quantities of seagrass and had high cellulase activity (0.02 ± 0.004 units mg^{-1}) to digest the constituent cellulose. *Petrolisthes elongatus* (porcelain crab) ingested brown and green phytoplankton and algae and had considerable laminarinase (0.35 ± 0.08 units mg^{-1}) and β -glucosidase (0.025 ± 0.005 units mg^{-1}) activities to digest the laminarin in its diet. *Leptograpsus variegatus* (omnivorous swift-footed shore crab) had high activities of protease (1.2 ± 0.02 units mg^{-1}), α -glucosidase, and α -amylase and appeared well equipped to utilize both dietary protein and carbohydrate. Stomach contents in *Nectocarcinus tuberculatus* (velvet crab) and *Carcinus maenas* (green crab) also suggest that these species are omnivorous. *N. tuberculatus* had high cellulase and chitinase for digesting the cellulose in plants and the chitin in invertebrate shells respectively. *C. maenas* had intermediate digestive enzyme levels and may employ more of a generalist feeding strategy than other species. *Plagusia chabrini* (speedy crab) is carnivorous, consuming encrusting bryozoans, hydroids, crustaceans, and fish. It has high protease activity, particularly trypsin

(0.73 ± 0.12 units mg^{-1}), to digest the protein in its animal prey. Each species of crab studied had a complex suite of digestive enzymes, the relative activities of which reflected individual and very different species-specific dietary niches.

Introduction

Crabs live in a variety of habitats with varying distributions and abundance of dietary items, so stomach contents typically include a diverse range of prey (Paul, 1981; Williams, 1982; Wear and Haddon, 1987). The variation in stomach contents between species from different habitats may reflect an opportunistic or versatile feeding nature where food items are consumed in proportion to their abundance in the surrounding habitat (Choy, 1986; Wolcott and Nancy, 1992), or it may indicate that crabs actively select habitat based on the presence of suitable food. Although most studies on the feeding habits of decapod crustaceans are based on the observation of stomach contents, stomach contents do not provide any information on dietary preference or the suitability of the diet for maintaining the animal. Similarly, stomach contents cannot help discriminate between generalist and targeted feeding strategies. Digestive enzymes however, may be a complementary tool useful for determining which dietary components are most effectively metabolized (Brêthes *et al.*, 1994). By understanding the digestion and assimilation of specific dietary components, we could identify the type of prey that the animals prefer and those that they are best equipped to digest. For example, carnivorous species exhibit a wide range and high activity of proteolytic enzymes to digest their high-protein diet, whereas herbivores and omnivores that ingest large amounts of carbohydrates possess highly active carbohydrases. Previous studies on the enzymatic system of decapod crusta-

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ceans have demonstrated this link between diet composition and the presence of digestive enzymes (Kristensen, 1972; Lee *et al.*, 1984; Johnston and Yellowlees, 1998; Hidalgo *et al.*, 1999; Figueiredo *et al.*, 2001).

The measurement of digestive enzyme synthesis is a tool commonly used to study trophic relationships in many invertebrate groups (McClintock *et al.*, 1991; Brêthes *et al.*, 1994). However, these studies have typically been limited to a few enzymes within one species (McClintock *et al.*, 1991; Brêthes *et al.*, 1994; Johnston and Yellowlees, 1998; Figueiredo *et al.*, 2001) or just one enzyme in a number of species (Galgani *et al.*, 1984). Our knowledge of crab digestive enzyme physiology is also limited. The few enzymes that have been documented include trypsin and carboxypeptidases A and B in *Callinectes sapidus* (blue crab) (Dendinger, 1987; Dendinger and O'Connor, 1990); α -amylase in *Carcinus maenas* (green crab) (Blandamer and Beechy, 1964); and α -glucosidase in *Cancer borealis* (jonah crab), *Cancer irroratus* (rock crab) (Brun and Wojtowicz, 1976), and *C. sapidus* (blue crab) (McClintock *et al.*, 1991). From a dietary perspective, Norman and Jones (1990) used activity of laminarinase as an indicator of the ability of *Liocarcinus puber* (velvet swimming crab) to utilize the brown algae frequently found in its stomach. Brêthes *et al.* (1994) used laminarinase and other enzymes as an index of trophic resource utilization by *Chionectes opilio* (snow crab).

The present study investigates stomach contents and digestive enzyme activities in six species of crab that inhabit a variety of habitats and specialized dietary niches. We examined the following crabs: *Nectocarcinus integrifrons* (Latreille) (red rock crab), *Petrolisthes elongatus* (Milne Edwards) (porcelain crab), *Leptograpsus variegatus* (Fabricius) (swift-footed shore crab), *Carcinus maenas* (Linnaeus) (green crab), *Plagusia chabrus* (Linnaeus) (speedy crab), and *Nectocarcinus tuberculatus* (Milne Edwards) (velvet crab). The objectives of this study were (1) to determine dietary preferences for each of the six crab species, using stomach content analysis, (2) to quantify the activities of a range of digestive proteases and carbohydrases in each crab species to determine how various food sources available to the crabs are utilized, and (3) to use dietary preferences and substrate utilization to help identify the position of the crabs in the trophic network of the coastal environment.

Materials and Methods

Collection

Crabs were collected from a number of sites in Tasmania by hand or by trapping. Collection was standardized to adults of each species during their periods of active feeding. When baited traps were used, the bait was positioned such that crabs could not ingest it and thereby bias the analysis of

stomach contents. *Nectocarcinus tuberculatus* was collected at night by scuba at Recherche Bay (43°0'S, 147°24'E) or in baited traps set overnight at Georges Bay (41°19'S, 148°15'E). *Petrolisthes elongatus* and *Leptograpsus variegatus* were collected by hand from the intertidal zone at Little Beach (41°31'S, 148°16'E) and in the Derwent River (42°53'S, 147°19'E) respectively. *Carcinus maenas* and *Nectocarcinus integrifrons* were collected in baited traps set overnight at Georges Bay (41°19'S, 148°15'E). *Plagusia chabrus* was collected at night from the research vessel RV *Challenger*, using baited traps at Great Oyster Bay (42°15'S, 148°16'E). Following collection, each crab was placed on ice for 10–20 min, after which its carapace, digestive gland, and stomach were removed. Digestive glands were frozen in liquid nitrogen and stored at –20 °C; stomachs were fixed in 10% formalin in 35 ppt seawater.

Stomach content verification

Each stomach was visually assessed for fullness (1 = empty, 2 = 25%, 3 = 50%, 4 = 75%, 5 = 100% full), and those with a score of 3 to 5 were dissected. The contents were examined using dissecting and compound microscopes and identified to the lowest possible taxonomic grouping by using appropriate keys. The stomachs of specimens of *L. variegatus* were not collected in this study, preventing a stomach content analysis.

Enzyme analysis

Individual digestive glands were thawed and homogenized for 5 min in chilled 100 mM Tris, 20 mM NaCl buffer, pH 7.0, using an UltraTurrax homogenizer. The homogenate was centrifuged at 968 g, and the supernatant containing digestive gland extract was transferred into microfuge tubes and stored at –20 °C.

Detailed procedures for enzyme assays are discussed elsewhere (Johnston, 2003). Briefly, total protease activity was measured using the casein hydrolysis method (Kunitz, 1947) as modified by Walter (1984) using tyrosine as the standard. Trypsin activity was measured using *N*- α -benzoyl-arginine- ρ -nitroanilide (BAPNA) as substrate using the molar absorption coefficient, ϵ , of $9300 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for ρ -nitroaniline (Stone *et al.*, 1991). α -Amylase activity was determined using the method of Biesiot and Capuzzo (1990), modified after Bernfeld (1955). α -Glucosidase, β -glucosidase, and chitinase activities were measured using the substrates ρ -nitrophenyl α -D-glucopyranoside, ρ -nitrophenyl β -D-glucopyranoside, and ρ -nitrophenol *N*-acetyl β -D-glucosaminide, respectively (Erlanger *et al.*, 1961). Cellulase activity was measured using the substrate sodium carboxymethyl cellulose (CM-cellulose). Laminarinase activity was measured using laminarin as the substrate.

We defined one enzyme unit (U) as the amount of en-

zyme that catalyzed the release of 1 μmol of product per minute, which we calculated using the appropriate molar extinction coefficient (ϵ) or a standard curve. Specific activity was defined as enzyme activity (U) per milligram of digestive gland protein (U mg protein^{-1}). Protein concentration was determined using the method of Bradford (1977), using bovine serum albumin as the standard. Enzyme assays were performed at 30 °C and the absorbances read using a TECAN Spectro Rainbow Thermo microplate reader (trypsin, α -amylase, α -glucosidase, β -glucosidase, chitinase) or a UNICAM 8625 UV/visible spectrophotometer (total protease, cellulase, laminarinase). Data points are the mean of duplicate assays accounting for the appropriate blanks, and each assay reports the mean \pm standard error of five replicate crabs for each species, with the exception of *N. tuberculosis*, for which 10 individuals were used.

Statistical analysis

Based on the activities for each enzyme within a species, differences between species were analyzed using a multivariate analysis of variance (MANOVA). Unlike univariate analyses, this analysis allows for the simultaneous comparison of species means for each enzyme while maintaining the chosen magnitude of type 1 error ($P = 0.05$) as well as considering the correlation between enzymes within a species. Following MANOVA, significant differences were explored using a canonical discriminant analysis (CDA). Each species was plotted in the reduced multivariate space, in which the new axes (CDA 1, CDA 2, and CDA 3) explain a proportion of the total variability in the data. Group

(species) centroids were plotted using the unstandardized canonical discriminant functions evaluated at group means, and each circle indicates the 95% confidence ellipses. Superimposed on this plot is the association between the new axes and the enzymes that were measured. This is displayed as a vector diagram in which the direction and length of the vector is a measure of the association between the enzyme and the axes. Those groups in which ellipsoids are not overlapping signify differences between species. The correlation between the position of each species relative to the vector diagram determines the enzyme or enzymes responsible for its separation.

Results

Stomach contents

Stomachs of all crabs had a large proportion of unidentifiable material that was either semi-digested or detritivorous in nature. Stomachs of *Nectocarcinus integrifrons* and *Petrolisthes elongatus* contained no animal material. *N. integrifrons* had large quantities of vascular plant material removed from either living or recently detached plants, whereas the stomachs of *P. elongatus* consisted largely of brown and green phytoplankton and larger algal pieces (Table 1). Stomachs of *Carcinus maenas* and *Nectocarcinus tuberculosis* had both plant and animal material (Table 1). Stomachs completely full of gastropod shells and bivalves were common in both crab species. Plant material was less common and consisted of small pieces of vascular material. Stomachs of *Plagusia chabrus* had very little identifiable

Table 1

Gut content items that were identified in the stomach of individual crabs with a stomach fullness greater than 3 (>50% full), and the corresponding diet from the literature

Species	Stomach content items		Literature diet	Reference	Classification
	Animal	Plant			
<i>Nectocarcinus integrifrons</i>	—	Vascular plant material	Feeds predominantly on the seagrass <i>Posidonia australis</i>	Klumpff & Nichols (1983)	Herbivore
<i>Petrolisthes elongatus</i>	—	Brown and green phytoplankton and algal pieces	Filter feeding — phytoplankton (e.g., diatoms); Deposit feeding — detritus	Achituv & Pedrotti (1999); Kropp (1981)	Herbivore
<i>Leptograpsus variegatus</i>	No stomachs collected		Limpets and barnacles; green and red algae	Skilleter & Anderson (1986); Griffin (1971)	Omnivore
<i>Carcinus maenas</i>	Molluscs	Vascular plant material	Bivalves, crustaceans, gastropods, and algae	Elner (1981)	Omnivore
<i>Nectocarcinus tuberculosis</i>	Molluscs, unidentifiable animal parts	Vascular plant material	—	—	Omnivore
<i>Plagusia chabrus</i>	Unidentifiable animal parts and small crustaceans	—	Encrusting animals — bryozoans, sponges, and hydroids Red algae and coralline algae	Edgar (2000) Griffin (1971)	Carnivore

plant matter and contained fragments of animal material, possibly small encrusting species of bryozoans and hydroids, as well as exoskeletons of small crustaceans and some fish parts (Table 1).

Digestive enzyme activity

Proteases. The highest protease activity was displayed by *Leptograpsus variegatus* (1.19 ± 0.02 units mg^{-1}) and *P. chabrus* (0.99 ± 0.05 units mg^{-1}). Lowest activity was measured in *N. integrifons* (0.34 ± 0.05 units mg^{-1}), *N. tuberculatus* (0.39 ± 0.02 units mg^{-1}), and *C. maenas* (0.46 ± 0.02 units mg^{-1}), with less than half the activity of *L. variegatus* and *P. chabrus* (Fig. 1A). The highest trypsin activity was exhibited by *P. chabrus* (0.73 ± 0.12 units mg^{-1}) and *L. variegatus* (0.46 ± 0.03 units mg^{-1}) and the lowest activity by *N. integrifons* (0.14 ± 0.03 units mg^{-1}) (Fig. 1B).

Carbohydrases. Carbohydrase activity was present in all crabs, with hydrolysis of both α - and β -linked substrates recorded in all species. α -Amylase activity was about three times higher in *Petrolisthes elongatus* (0.29 ± 0.04 units mg^{-1}) than in *N. tuberculatus* (0.09 ± 0.02 units mg^{-1}) (Fig. 2A). α -Glucosidase specific activity was highest in *L. variegatus* (0.0022 ± 0.0002 units mg^{-1}) and was about twice the level recorded for all other species. *Petrolisthes elongatus* had negligible α -glucosidase activity (Fig. 2B).

β -Glucosidase activity was highest in *P. elongatus* (0.025 ± 0.005 units mg^{-1}), about three times greater than in *L. variegatus* (0.007 ± 0.0008 units mg^{-1}) (Fig. 3A). Although variable, laminarinase activity was highest in *P. elongatus* (0.35 ± 0.08 units mg^{-1}), and *L. variegatus* also had substantial activity (0.18 ± 0.02 units mg^{-1}) (Fig. 3B). The other four species had comparatively lower activity, ranging between 0.058 ± 0.01 (*N. tuberculatus*) and 0.016 ± 0.003 (*C. maenas*) units mg^{-1} . Cellulase activity was highest for *N. integrifons* (0.019 ± 0.004 units mg^{-1}) and lowest in *C. maenas* (0.0014 ± 0.0006 units mg^{-1}) and *Plagusia chabrus* (0.0019 ± 0.0014 units mg^{-1}) (Fig. 4A). Chitinase activity was similar for most species, ranging between 0.023 ± 0.003 (*P. chabrus*) and 0.041 ± 0.005 (*L. variegatus*) units mg^{-1} . The exception was *Petrolisthes elongatus*, which had a substantially lower chitinase activity (0.006 ± 0.001 units mg^{-1}) (Fig. 4B).

Relationship between enzyme complement and crab species

Significant differences were found between crab species when the specific activities of all enzymes were compared using MANOVA (Pillai's Trace = 3.347; $F_{(40,130)} = 6.581$, $P < 0.001$). The CDA explained 72.2% of the variation on the first and second axes (CDA 1 and CDA 2) and 39.6% on the second and third axes (CDA 2 and CDA 3) (Figs. 5 and

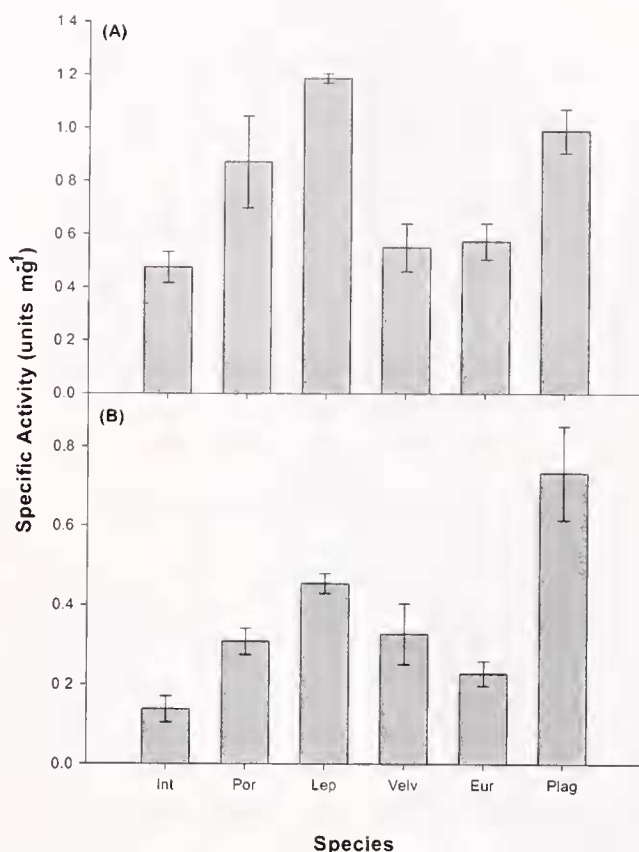


Figure 1. Specific activity of (A) total protease and (B) trypsin measured from crude digestive gland extract of six crab species with different feeding habits. Values are mean (units mg^{-1}) \pm S.E. where units are in $\mu\text{mol } p\text{-nitroanilide min}^{-1}$ for trypsin and $\mu\text{g tyrosine min}^{-1}$ for total protease. Key to crab species: Int = *Nectocarcinus integrifons* ($n = 5$), Por = *Petrolisthes elongatus* ($n = 5$), Lep = *Leptograpsus variegatus* ($n = 5$), Velv = *Nectocarcinus tuberculatus* ($n = 10$), Eur = *Carcinus maenas* ($n = 5$), Plag = *Plagusia chabrus* ($n = 5$).

6). The greatest difference among the species was along the first axis, CDA 1 (x axis), which explained 51% of the variation (Fig. 5). This difference was largely due to separation between the species on the basis of the high activity of laminarinase and β -glucosidase in *P. elongatus*, and the high activity of α -glucosidase displayed by *L. variegatus*. The second axis, CDA 2 (y axis) also showed differences between the species and accounted for 21.2% of variation, with *Plagusia chabrus* being separated from other species by its high activity of trypsin and total protease, while *N. integrifons* and *N. tuberculatus* were separated by their activity of cellulase and chitinase (Fig. 5). The third axis (CDA 3) explained 18.4% of the variation and, when plotted with CDA 2, shows a separation between *N. integrifons* and *N. tuberculatus* (Fig. 6). These two species are still separated along CDA 2 by their cellulase and chitinase activity, but are now separated from each other along CDA 3 by the higher activity of cellulase in *N. integrifons* and the higher

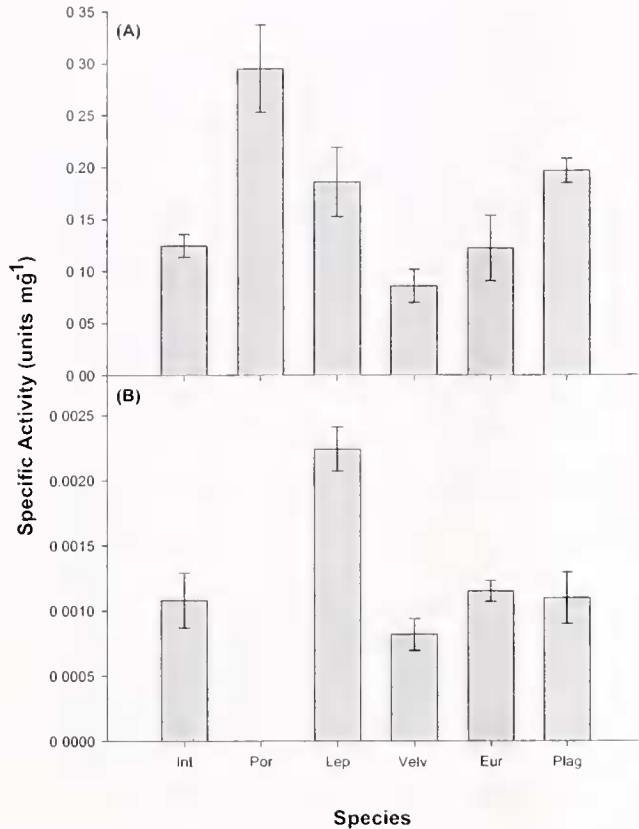


Figure 2. Specific activity of (A) α -amylase and (B) α -glucosidase measured from crude digestive gland extract of six crab species with different feeding habits. Values are mean (units mg^{-1}) \pm S.E. where units are in μmol maltose min^{-1} for α -amylase and in μmol *p*-nitrophenol min^{-1} for α -glucosidase. Key to crab species: Int = *Nectocarcinus integrifrons* ($n = 5$), Por = *Petrolisthes elongatus* ($n = 5$), Lep = *Leptograpsus variegatus* ($n = 5$), Velv = *Nectocarcinus tuberculatus* ($n = 10$), Eur = *Carcinus maenas* ($n = 5$), Plag = *Plagusia chabrus* ($n = 5$).

trypsin activity of *N. tuberculatus*. The *C. maenas* lies central on both plots, only being separated in Figure 6 by its relative activity of α -glucosidase.

Discussion

We investigated the dietary preference of six species of crab on the basis of their stomach contents and used analysis of digestive enzymes to determine which dietary components are most likely being assimilated. Digestive enzyme activities are an effective tool for identifying particular components of an animal's diet. High proteolytic activity reflects a diet high in protein, high carbohydrase activity reflects a diet high in starch or cellulose, and high lipase activities reflect a diet high in fat (Lee *et al.*, 1984; Johnston and Yellowlees, 1998; Johnston, 2003). Multivariate analyses (MANOVA) simultaneously compared the relative activities of proteases and carbohydrases within each species, as well as individual enzymes between spe-

cies, and separated the six crab species into separate dietary preferences. For each species, one enzyme (*i.e.*, laminarinase, β -glucosidase, cellulase, trypsin, total protease, and α -glucosidase) was responsible for separating each species from the other five (Figs. 5, 6), suggesting that each species occupies a different dietary niche.

Plagusia chabrus (speedy crab)

Stomachs of *P. chabrus* contained fragments of animal material, possibly of small encrusting species of bryozoans and hydroids (see Edgar, 2000) (Table 1). *Plagusia chabrus* had very little identifiable plant matter within its stomach and appears to be almost totally carnivorous. However, red and coralline algae have previously been found in the stomach of this species (Griffin, 1971). This apparent discrepancy may be due to differences in the abundance and structure of potential prey communities (Paul, 1981; Haefner, 1990; Freire, 1996). Stomach contents of *P.*

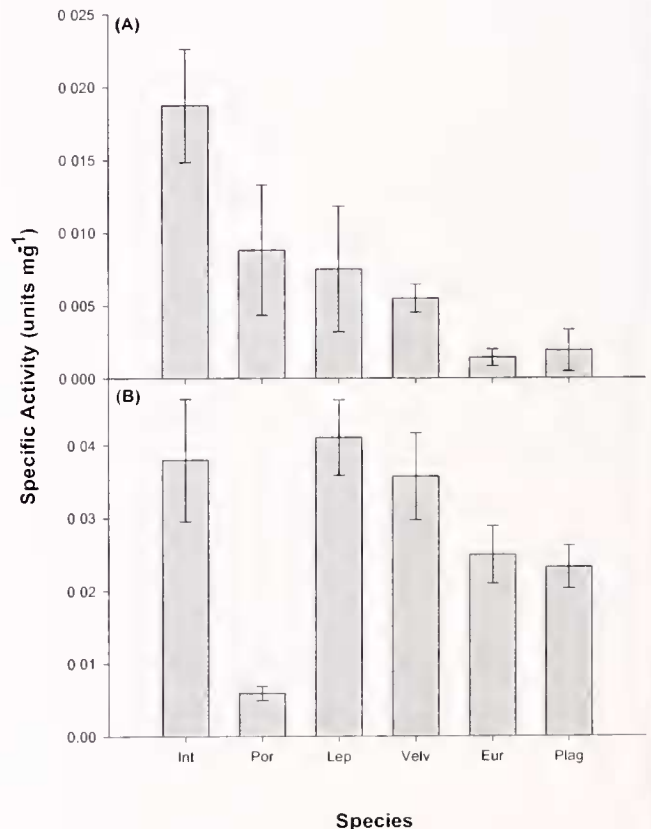


Figure 3. Specific activity of (A) cellulase and (B) chitinase measured from crude digestive gland extract of six crab species with different feeding habits. Values are mean (units mg^{-1}) \pm S.E., where units are in μmol glucose min^{-1} for cellulase and in μmol *p*-nitrophenol min^{-1} for chitinase. Key to crab species: Int = *Nectocarcinus integrifrons* ($n = 5$), Por = *Petrolisthes elongatus* ($n = 5$), Lep = *Leptograpsus variegatus* ($n = 5$), Velv = *Nectocarcinus tuberculatus* ($n = 10$), Eur = *Carcinus maenas* ($n = 5$), Plag = *Plagusia chabrus* ($n = 5$).

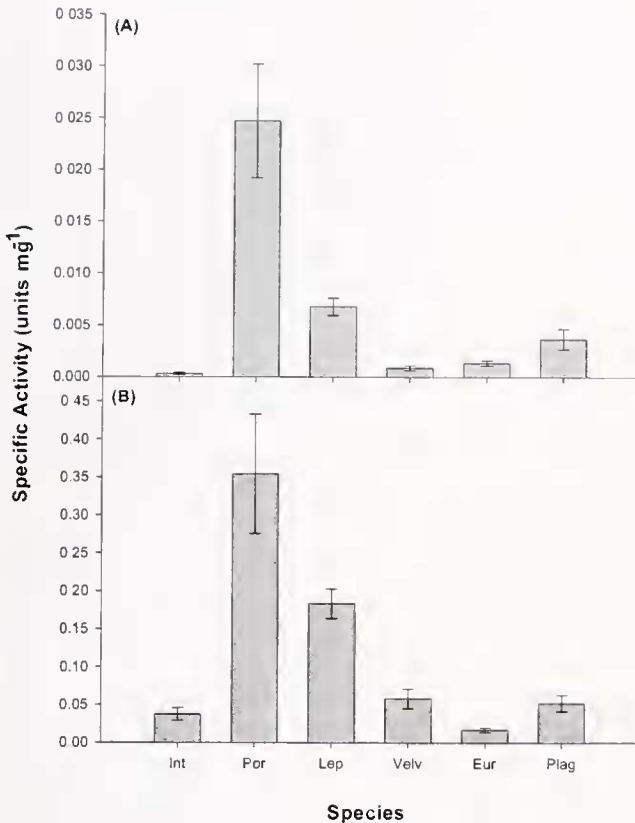


Figure 4. Specific activity of (A) β -glucosidase and (B) laminarinase measured from crude digestive gland extract of six crab species with different feeding habits. Values are mean (units mg^{-1}) \pm S.E., where units are in $\mu\text{mol } p\text{-nitrophenol min}^{-1}$ for β -glucosidase and in $\mu\text{mol glucose min}^{-1}$ for laminarinase. Key to crab species: Int = *Nectocarcinus integrifrons* ($n = 5$), Por = *Petrolisthes elongatus* ($n = 5$), Lep = *Leptograpsus variegatus* ($n = 5$), Velv = *Nectocarcinus tuberculatus* ($n = 10$), Eur = *Carcinus maenas* ($n = 5$), Plag = *Plagusia chabrus* ($n = 5$).

chabrus collected in this study were similar to those observed by Edgar (2000). In both studies, crabs were sampled subtidally from exposed reef in 3–5 m of water. In contrast, the majority (72%) of *P. chabrus* captured by Griffin (1971) were collected from exposed rocky platforms in the intertidal zone where red and coralline algae are more common. The greater abundance of these algae may make them a more attractive food source or more likely to be incidentally ingested.

Reflecting its carnivorous nature, *P. chabrus* displayed high protease and trypsin activity, and it was this high activity of protease and trypsin that separated *P. chabrus* from the other crab species in the MANOVA. *P. chabrus* had the highest trypsin activity encountered in any of the species studied. The trypsin activity was similar in value to that of the slipper lobster, *Thenus orientalis* (0.54 units mg^{-1}), and the karuma prawn, *Penaeus japonicus* (0.73 units mg^{-1}), two species whose diets also contain consid-

erable amounts of protein (Maugle, 1982; Johnston and Yellowlees, 1998) (Table 2).

The low plant content in the diet of *P. chabrus* is reflected by its low activity of cellulase, an enzyme that breaks down the cellulose in plants. The high α -amylase activity is likely to reflect the high glycogen content of animal prey consumed by *P. chabrus*. Similarly high amylase activity in the carnivorous American lobster *Homarus americanus* (1.5 units mg^{-1}) also reflects digestion of glycogen in animal prey (Wojtowicz and Brockerhoff, 1972; Table 2). However, α -amylase is also involved in the digestion of starch in plant tissue (see *P. elongatus* below), so the relationship between amylase activity and carnivory is not ubiquitous.

Leptograpsus variegatus (swift-footed shore crab)

Two comprehensive dietary studies of *L. variegatus* revealed a mixture of plant material (the green alga *Ulva lactuca*, the coralline alga *Corallina officinalis*, and the red algae *Polysiphonia* sp. and *Ceramium* sp.) and animal material (limpets and barnacles) within its stomach (Skilleter and Anderson, 1986; Griffin, 1971). These studies suggest that *L. variegatus* is an omnivore that actively scavenges in the intertidal zone for invertebrates and algae (Table 1).

We found *L. variegatus* to have the highest protease activity of all species studied, an observation not immedi-

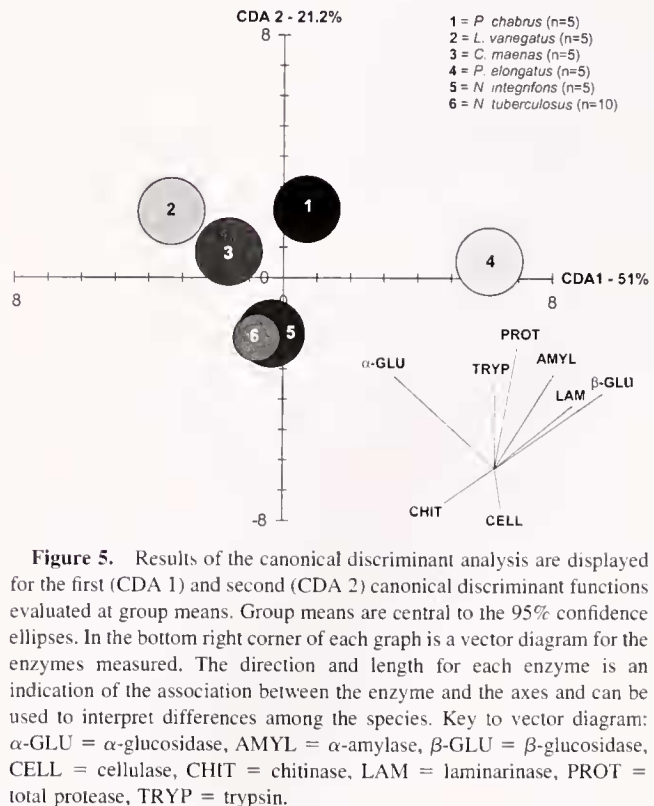


Figure 5. Results of the canonical discriminant analysis are displayed for the first (CDA 1) and second (CDA 2) canonical discriminant functions evaluated at group means. Group means are central to the 95% confidence ellipses. In the bottom right corner of each graph is a vector diagram for the enzymes measured. The direction and length for each enzyme is an indication of the association between the enzyme and the axes and can be used to interpret differences among the species. Key to vector diagram: α -GLU = α -glucosidase, AMYL = α -amylase, β -GLU = β -glucosidase, CELL = cellulase, CHIT = chitinase, LAM = laminarinase, PROT = total protease, TRYP = trypsin.

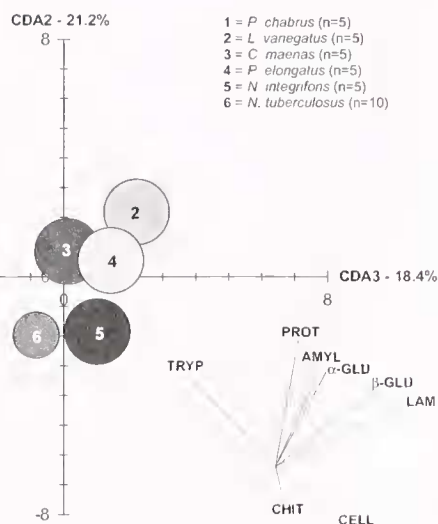


Figure 6. Results of the canonical discriminant analysis are displayed for the second (CDA 2) and third (CDA 3) canonical discriminant functions evaluated at group means. Group means are central to the 95% confidence ellipses. In the bottom right corner of each graph is a vector diagram for the enzymes measured. The direction and length for each enzyme is an indication of the association between the enzyme and the axes and can be used to interpret differences among the species. Key to vector diagram: α -GLU = α -glucosidase, AMYL = α -amylase, β -GLU = β -glucosidase, CELL = cellulase, CHIT = chitinase, LAM = laminarinase, PROT = total protease, TRYP = trypsin.

ately consistent with an omnivorous feeding strategy. Although it is generally accepted that high protease activity reflects a carnivorous diet, other studies have also found high proteolytic activity in omnivores (Jonas *et al.*, 1983; Hidalgo *et al.*, 1999). The sea lettuce (*Ulva lactuca*), which is ingested in large quantities by *L. variegatus* (Lobban and Harrison, 1997), is high in protein (15% of the organic matter) and may contribute to the high protease activity in this crab.

More in line with an omnivorous diet, the α -glucosidase activity was about twice as high in *L. variegatus* as in any other species we studied. This high α -glucosidase activity was responsible for separating *L. variegatus* from all other crab species in the MANOVA and, coupled with substantial α -amylase activity, suggests that *L. variegatus* is well equipped to utilize the carbohydrates within its diet. The strong activity of α -enzymes indicates that α -linked storage carbohydrates are important in its diet. Such storage products are present in both green algae (*i.e.*, starch, which is a mixture of amylose and amylopectin) and red algae (*i.e.*, floridean starch, a branched glucan similar to amylopectin) (Lobban and Harrison, 1997). *L. variegatus* also had substantial laminarinase activity, which suggests that this crab may also ingest brown algae, a component not described in previous dietary studies (Griffin, 1971; Skilleter and Anderson, 1986).

Carcinus maenas (green crab) and *Nectocarcinus tuberculosa* (velvet crab)

Carcinus maenas and *N. tuberculosa* had both plant and animal (gastropod shells and bivalves) material within their stomachs (Table 1), suggesting they are omnivores. *Carcinus maenas* is well studied and is described as a voracious predator feeding primarily on bivalve molluscs, polychaetes, and small crustaceans (Elner, 1981; MacKinnon, 1997) (Table 1). However, algae have also been frequently observed in its stomach but generally do not contribute more than about 10% to the total volume of stomach contents (Elner, 1981). The diet of *N. tuberculosa* has not been studied, but the morphology of its mouthparts, in particular the specialized mandibles, appears suited to grinding hard animals (such as molluscs) and vascular plant material (Salindeho and Johnston, 2003).

Although our stomach content analysis suggests both species to be omnivorous, their digestive enzyme complement differs from that of the omnivorous *L. variegatus*. Protease activity for both *N. tuberculosa* and *C. maenas* was less than half that of *L. variegatus*. This may be due to a reliance on plant material with lower protein content than the sea lettuce consumed by *L. variegatus*. The protease levels of *N. tuberculosa* and *C. maenas* are similar to that of the scavenging omnivorous redclaw crayfish *Cherax quadricarinatus* (0.236 units mg^{-1}) (Figueiredo *et al.*, 2001) (Table 2).

Despite the plant material in the stomach of these species, laminarinase activity was low, suggesting that brown algae are not an important dietary component of either species. In the MANOVA, *N. tuberculosa* is separated from the other crab species by its cellulase and chitinase activity, which reflects its ability to break down and digest plant cellulose as well as the chitinous shells of molluscs and other invertebrates. Interestingly, *C. maenas* lies fairly centrally on the axis of both CDA plots (Figs. 5, 6), which suggests they have intermediate levels of all digestive enzymes compared to the other crab species. *C. maenas* may be more of a generalist feeder, utilizing a broader spectrum of dietary items. Such a strategy would help to explain its incredible success in a wide range of habitats (Cohen *et al.*, 1995).

Nectocarcinus integrifrons (red rock crab)

Herbivory is common in crabs—the consumption of vascular plants has been observed in several species (Giddins *et al.*, 1986; Kyomo, 1992; Woods and Schiel, 1997). The stomachs of *N. integrifrons* contained no animal material but did contain large quantities of vascular plant material (Table 1). Klumpp and Nichols (1983) found the seagrass *Posidonia australis* to occur in the stomachs of 93% of the *N. integrifrons* individuals sampled and to occupy 85% of stomach volume. Consistent with a diet low in protein (seagrass is only 7% protein), protease and trypsin activities were

Table 2

Comparison of specific activities of crustacean proteases and carbohydrases from crude digestive gland extracts

Species	Enzyme								Reference
	Prot	Tryp	α -Am	α -Glu	β -Glu	Cell	Lam	Chit	
<i>Nectocarcinus integrifrons</i>	0.47	0.14	0.12	0.0010	0.0003	0.019	0.037	0.038	This study
<i>Petrolisthes elongatus</i>	0.87	0.31	0.29	Neg	0.0250	0.009	0.35	0.006	
<i>Leptograpsus variegatus</i>	1.2	0.46	0.19	0.0022	0.0067	0.007	0.18	0.041	
<i>Nectocarcinus tuberculatus</i>	0.55	0.33	0.09	0.0008	0.0009	0.005	0.058	0.036	
<i>Carcinus maenas</i>	0.57	0.23	0.12	0.0012	0.0013	0.001	0.017	0.025	
<i>Plagusia chabrus</i>	0.99	0.73	0.20	0.0011	0.0037	0.002	0.052	0.023	
Crabs									
<i>Carcinus maenas</i>			1.2						Blandamer & Beechy (1964)
<i>Chionoecetes opilio</i>			0.10						Brêthes <i>et al.</i> (1994)
<i>Callinectes sapidus</i>				0.0096	0.01				McClintock <i>et al.</i> (1991)
<i>Cancer irroratus</i>			0.24	0.001	0.008			0.23	Brun & Wojtowicz (1976)
<i>Cancer borealis</i>			0.12	0.001	0.009			0.09	Brun & Wojtowicz (1976)
Prawns									
<i>Penaeus vannamei</i>				0.07					Le Chevalier & Van Wormhoudt (1998)
<i>P. monodon</i>			0.13				0.45		Wigglesworth & Griffith (1994)
<i>P. keratulus</i>		0.060							Galgani <i>et al.</i> (1984)
<i>P. japonicus</i>		0.057							Galgani <i>et al.</i> (1984)
<i>P. japonicus</i>		0.73							Maugle (1982)
<i>P. indicus</i>				0.016	0.014		0.036		Omondi & Stark (1995)
Lobsters and Crayfish									
<i>Homarus americanus</i>			1.5						Wojtowicz & Brockerhoff (1972)
<i>Thenus orientalis</i>		0.54		0.022	0.004	Neg		0.42	Johnston & Yellowlees (1998)
<i>Cherax quadricarinatus</i>		0.236	0.821	0.11	0.041		0.12	0.15	Figueiredo <i>et al.</i> (2001)
<i>C. quadricarinatus</i>						0.03			Xue <i>et al.</i> (1999)

Prot = Total protease; Tryp = Trypsin; α -Am = α -Amylase; α -Glu = α -Glucosidase; β -Glu = β -Glucosidase; Cell = Cellulase; Lam = Laminarinase; Chit = Chitinase. Substrates and units are identical with those used in this study, with values in units mg^{-1} where units are $\mu\text{mol min}^{-1}$ except for protease, which is $\mu\text{g tyrosine min}^{-1}$. Neg. = no activity detected.

lower in *N. integrifrons* than in the other species. *Nectocarcinus integrifrons* had the highest level of cellulase activity, and it is this enzyme that is responsible for separating this crab from other species in the MANOVA. Cellulase is required to digest cellulose, the primary structural component of vascular seagrass. Klumpp and Nichols (1983) also found high cellulase activity in both the digestive gland and stomach contents of *N. integrifrons*. Using chemical analysis, they determined that with combined enzymatic and mechanical action, *N. integrifrons* is able to digest up to 40% of ingested plant fiber (Klumpp and Nichols, 1983). Red-claw crayfish *C. quadricarinatus* also consumes significant amounts of plant material and has cellulase activity compa-

table to that of the red rock crab (0.03 units mg^{-1}) (Xue *et al.*, 1999).

Petrolisthes elongatus (porcelain crab)

Like *N. integrifrons*, *P. elongatus* is herbivorous. Stomach contents contained no traces of animal matter and consisted largely of brown and green phytoplankton and larger algal pieces. Porcelain crabs are able to use their setose third maxillipeds to filter plankton from the water column (Caine, 1975). Although zooplankton could be captured in this fashion, they were not evident in the stomachs of crabs sampled. Alternative feeding methods, such as direct use of

the chelipeds to chop pieces of algae for ingestion or the feeding on detritus, may account for the occurrence of multicellular algae and detritus in the stomachs of *P. elongatus* (Kropp, 1981).

Surprisingly, the herbivorous *P. elongatus* had high total protease activity (Table 2). There are two explanations for this. Firstly, high protease activity may be a physiological adaptation to maximize digestion of small amounts of protein from large volumes of ingested plankton. Microphagous feeding in adult porcelain crabs is similar to planktivorous (phytoplankton) feeding by larval crustaceans. Comparative studies on the digestive enzymes of crustacean larvae indicate that protease activities may be higher in animals that consume phytoplankton than in carnivorous larvae. High protease activity may enable these species to rapidly extract the relatively small protein component from large volumes of food, so there is a net energy gain despite a relatively low overall assimilation efficiency (Kumlu and Jones, 1997; Le Vay *et al.*, 2001). Secondly, it is possible that *P. elongatus* is actually omnivorous and that zooplankton could have been ingested during filter feeding. However, zooplankton was not identified in the stomachs of animals sampled in this study. Furthermore, *P. elongatus* had substantially lower chitinase activity than the other species studied here, suggesting a poor capacity to break down chitin, a structural component of the exoskeleton of zooplankton and other invertebrates. Omnivorous species that do ingest shelled invertebrates, such as *L. variegatus* and *N. tuberculosus*, possess considerable chitinase performance.

The activities of the carbohydrases (indicative of plant digestion) were mixed, giving us an insight into the specific carbohydrates assimilated by *P. elongatus*. α -Amylase activity was very high, about three times higher than in *N. tuberculosus*. High α -amylase activity reflects the high proportion of starch in plants ingested by *P. elongatus* (Sabapathy and Teo, 1993). Interestingly, α -glucosidase activity was negligible, which suggests that although *P. elongatus* is highly efficient at digesting large structural polysaccharides such as starch using α -amylase, it is less effective at digesting smaller oligosaccharides, which are broken down using α -glucosidase.

β -Glucosidase activity was highest in the porcelain crab—about three times greater than in the next highest species, *L. variegatus*. Laminarinase, an enzyme complex that includes exo- and endo-hydrolytic β -1,3 glucanases as well as β -glucosidase, was also the highest in *P. elongatus*. It was this high laminarinase and β -glucosidase activity that separated *P. elongatus* from all other species in the MANOVA. Laminarin is a β -1,3-linked polymer of glucose stored in brown algae (Lobban and Harrison, 1997). The laminarinase and β -glucosidase enzyme combination in *P. elongatus* is ideally suited for digesting the types of algae found within the gut of this species, and these enzymes

appear suitable as indicators of the dietary preference for brown algae (Figueiredo *et al.*, 2001; Wigglesworth and Griffith, 1994).

Conclusions—digestive enzyme complement as indicator of diet type

Each species of crab studied had a complex suite of digestive enzymes, the relative activities of which reflected species-specific dietary niches. As opportunistic feeders, crabs have a wide range of digestive enzymes. However, it is clear from this study that the specific enzymes dominant within each crab species are consistent with their particular diets. The porcelain crab *P. elongatus* has high activities of laminarinase and β -glucosidase for digesting dietary brown algae (laminarin). High cellulase activity is necessary to digest the vascular seagrass (cellulose) diet of the red rock crab *N. integrifrons*. Significant trypsin and total protease activities break down the high-protein diet of the speedy crab *P. chabrus*. For the swift-footed shore crab *L. variegatus*, digestion of the starch in its predominantly red and green algal diet is achieved via high α -glucosidase and α -amylase activities. The velvet crab *N. tuberculosus* has high cellulase activity to digest the cellulose of its plant diet and high chitinase activity to digest the chitinous shells of the molluscs and other invertebrates that it also consumes. The specific nature of the enzymes in most crab species encountered here appears to favor a specific feeding behavior and dietary preference, and demonstrates different strategies of resource use. In contrast to the other species, the green crab *C. maenas* did not appear to have a dominant enzyme, which suggests that it is a generalist feeder that utilizes a broad range of dietary items, which may help to explain its incredible success in a range of diverse habitats.

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